N° 1

LECTIN-REACTIVE a-FETOPROTEIN IN THE LIVER TRANSPLANT ASSESSMENT OF PATIENTS WITH TYROSINAEMIA TYPE I

U. Baumann*, V. Duhme, P.J. McKiernan and E. Holme. Birmingham Children's Hospital, Birmingham, UK. **With permission of the participants of the NTBC study group.*

Despite the introduction of NTBC into the treatment of tyrosinaemia type I (TTI) hepatocellular carcinoma (HCC) does occur in affected patients. Serial total α-Fetoprotein (AFP) levels are used to evaluate the individual risk to develop malignant changes. Lectin-reactive α-Fetoprotein is a recently described marker for early recognition of HCC in adult liver disease.

Aim : To investigate if the analysis for Lectin-reactive α-Fetoprotein could lead to earlier detection of HCC compared to a judgement based on the evolution of total AFP alone.

Patients : We report the analysis of AFP data from 41 patients with TTI. 12 patients had TTI and histologically proven HCC.

Methods : AFP electrophoresis in a lectin containing agarose gel leads to separation of lectin-reactive and non-lectin-reactive AFP fractions. A proportion of more than 15% of lectin-reactive AFP is suspicious for the development of HCC in adult cirrhotic patients.

Results : AFP subfractions could be identified in all 41 patients. In the patients with TTI and HCC in 6 patients the lectin reactive AFP was elevated before total AFP became abnormal, in 3 patients the rise in lectin reactive-AFP was consistant with the rise of the total AFP and in 3 patients the lectin reactive-AFP was raised after the total AFP or did not increase at all.

Discussion : We were able to identify 6 out of 12 patients with HCC who had an early rise of lectin reactive AFP before they developed a change in total AFP levels.

Conclusions : Analysis for lectin reactive AFP may allow early diagnosis of HCC in patients with TTI and thus facilitate liver transplantation before the onset of metastasis. We suggest the further evaluation of lectin-reactive AFP in TTI.

N° 2

IMMUNOLOGICAL CONSIDERATIONS IN LIVER CELL TRANSPLANTATION FOR CRIGLER-NAJJAR SYNDROME

Katrina Allen[1,5], Daphne Cheah[1], Paul Wright[2], Mark Brooks[2], Peter Angus[2], Robert Jone[3], Robert Williamson[4], Winita Hardikar[5]. (1) Murdoch Childrens Research Institute ; (2) Royal Melbourne Institute of Technology ; (3) Victorian Liver Transplant Unit Austin Hospital ; (4) University of Melbourne ; (5) Department of Gastroenterology Royal Children's Hospital, Melbourne, Australia.

The aims were to monitor immune responses post-liver cell transplantation (LCT) for a patient with Crigler-Najjar syndrome type 1. Fresh human hepatocytes were from an ABO-matched female cadaveric donor by a standard-2-step collagenase perfusion. Cell viability was 93% and overnight plating efficiency was >90%. The 8 year old female received $1x10^7$/ml liver cell suspension via a 5 French radiologically placed portal catheter. Immunosuppression was tacrolimus, azathioprine and prednisolone. ELISA screen for IgG antibodies to HLA Class I and II antigens (GTI Quick-ID and GTI B-Screen) was performed monthly for 18 months prior to and 5 months after LCT. CDC was performed on 4 occasions prior to the transplant and at the time of transplant using donor lymphocytes. The transplanted cells were functioning as evidenced by a significant reduction in serum bilirubin from 360 μmol/L while maintaining pre-transplant phototherapy times of 8 hours per day. Function was maintained as phototherapy was reduced and eventually halved to 4 hours. From 80 days post-LCT, there was a lost of cell function with serum bilirubin rising to pre-transplant levels on maximal phototherapy of 8 hours. A positive CDC to 2 different panels of HLA class I antigen (15% and 17%) was detected 18 months prior to LCT, but became negative on subsequent testing on 3 occasions. CDC using donot T and B cells against recipient serum was also negative at the time of LCT. The recipient had a negative ELISA screen for IgG antibodies to HLA Class I and II antigens monthly for 18 months prior to LCT. Despite a complete antigen mismatch for HLA A, B and DR between donor and recipient, ELISA remained negative when tested 5 months post LCT. Liver cell transplantation is a safe and effective method for augmenting enzyme function in a metabolic liver disease. Loss of cell function does not appear to be related to HLA sensitization.

N° 3

HEPATOCELLULAR TUMORS IN METABOLIC DISORDERS

Richter A., Grabhorn E., Ganschow R., Burdelski M. Pediatric Gastroenterology and Hepatology, University Medical Center Hamburg-Eppendorf, Germany.

Objective : Metabolic disorders such as tyrosinemia are known to have a high risk of developing hepatocellular carcinomas. We report two cases of hepatocellular tumors in children with tyrosinemia type I. Furthermore, hepatic malignancies were detected incidentally in two patients with Progressive Familial Intrahepatic Cholestasis type 2 (PFIC2) undergoing liver transplantation (OLT) for end-stage cirrhosis.
Patients : Patient one and two suffered from tyrosinemia type I and underwent nitisinone therapy. Despite this therapy, a hepatocellular carcinoma (case one) and a hepatoblastoma (case two) were diagnosed by radiological methods. The children underwent OLT. Patient three and four underwent liver transplantation for end-stage cholestatic liver cirrhosis due to PFIC2. Incidentally, a hepatoblastoma (case three) and a hepatocellular carcinoma (case four) were detected during the operative procedure.
Results : From 1986 to 2005, 3 patients with tyrosinemia underwent OLT in our center. Two children suffered from hepatocellular tumors (66%). 19 patients with PFIC2 were transplanted in our center. In 2 patients, a hepatocellular tumor was diagnosed incidentally (11%). It had not been detected on ultrasound examination of the cirrhotic liver. Alpha-fetoprotein (AFP) was found to be very high prior to transplantation in all cases. Because of the absence of metastasis, none of the children received chemotherapy. After a period of 3-5 years, there are no signs of disease recurrence detectable by clinical and radiological methods.
Conclusion : Metabolic disorders like tyrosinemia bear a high risk of developing a hepatocellular carcinoma. Progressive Familial Intrahepatic Cholestasis seems to predispose to hepatic malignancies as well. Routine screening should include an ultrasound examination, the quantification of AFP and histological examination of the native liver to exclude malignancies.

N° 4

SEQUELAE OF UNDELRYING DISEASE AND POSTTRANSPLANT COMPLICATIONS IN LIVER TRANSPLANTATION FOR METABOLIC DISORDERS

Beckmann, D. Broering, L. Fischer, C. Hillert, K. Helmke, K. Ullrich, X. Rogiers. Dept Pediatrics, Pediatric Radiology and Hepatobiliary Surgery, University Medical Center Hamburg-Eppendorf.

Sinde 10 year patient survival in pediatric LTX has reached 80%, risks and benefits of this therapy in hepatic metabolic disorders must be reevaluated. We have compared metabolic and posttransplant complications in 70 patients transplanted in our center for metabolic disorders between X 1991 and VI 2004 : PFIC2 (n =18), PFIC3 (n=11), Crigler-Najjar syndrome (n=10), Oxaluria (n=8), α1ATD (n=6), Wilson's disease (n=4), Tyrosinemia I (n=3), Respiratory chain disorders (n=3), Glycogen Storage Disease (n=2), Cystic Fibrosis (n=2), Urea Cycle defects (n=2) and Niemann Pick C (NPC), (n=1). Diagnosis fo RC and NPC were only made in retrospect. Survival ranged from 0% (RCD, NPC), 50% (WD), 73% (Oxaluria), 81% (PFIC2), 83% (αATD) to 100% (PFIC3, CN, Ty, UCD, CF and GSD IV). Graft loss due to chronic rejection (n=6) and primry non function (n=4) were the cause of reLTX in 10 patients. Death related to posttransplant complications were seen in PFIC2 (n=4), Oxaluria (n=2), 1αATD and WD (n=1). In the ladt 5 years, no death from posttransplant complications were observed. On the other hand death from complications of the underlying disease were observed in all RCD patients and in NPC and WD (1/4). Morbidity related to the underlying disease were cerebral convulsion (CN, n=2) and disease recurrence in PFIC2 (2/18). Renal insufficiency occurred in 3/8 patients with Oxaluria. Quality of life improved dramatically without need for diet in Urea Cycle defects and Ty1, no itching in PFIC2 and 3 and no need for hospitalisation except for quality controls in all patients without complications. With these results, liver transplantation should be considered as a good option in hepatic based metabolic disorders. Early referral helps to avoid disease related complications. Preemptive LTX should be considered. Correct diagnosis may be difficult in patients presenting with acute liver failure.

N° 5

DOES LONG-TERM PHOTOTHERAPY AFFECT THE LIVER IN PATIENTS WITH CRIGLER-NAJJAR SYNDROME ?

Deutschmann A.[1], Ganschow R.[2], Schäfer H.[2], Burdelski M.[2] (1) Department of Paediatrics, Medical Universitiy of Graz ; (2) Department of Paediatrics, University Hospital Hamburg-Eppendorf.

Introduction : Crigler-Najjar syndrome (CNS) is characterised by unconjugated hyperbilirubinemia since birth due to a deficiency of the enzyme bilirubin-UDP-glucuronosyl transferase. Fatal bilirubin encephalopathy may develop unpredictably. Therapy consists of long-term phototherapy with or without phenobarbital treatment until liver transplantation (LTx).

Patients and methods : A retrospective study was performed. 9 children were diagnosed for CNS type 1, and 3 children were diagnosed for CNS type 2. All patients received a liver transplantation. Liver function tests, bile acids, virology and histology of the explanted livers were evaluated.

Results : The average time of daily phototherapy until LTx was 11 hours per day. Median cumulative time of phototherapy was 6.5 years (range 0.6-19.2 years). Phenobarbiton was administered to nine children. Increased pretransplant liver function tests were shown in nine children. There was no correlation between duration of phototherapy and elevation of liver enzymes. Viral infection was excluded. Bile acids were normal. Histology of the explanted livers showed centrolobular cholestasis (n = 11) and additionally focal, reticular centrolobular fibrosis (n = 4).

Discussion : Increased serum liver enzymes and gammaglutamyl transpeptidase levels, normal values for bile acids in combination with the histological pattern of centrolobular cholestasis and focal, centrolobular fibrosis could be indicative for toxic liver damage. These findings also were observed in patients without phenobarbital treatment. Therefore the reason is unclear. Long-time phototherapy is exclusively performed in patients with CNS. The question is, if intermediate photoproducts resulting from phototherapy, in a long-time manner contribute to liver injury?

However, in conclusion, liver injury apart from the steady risk of developing kernicterus is another argument favouring early liver transplantation in patients with Crigler-Najjar syndrome.

N° 6

LONG-TERM RESULTS OF PRE-EMPTIVE LIVER TRANSPLANTATION IN PRIMARY HYPEROXALURIA TYPE 1

M. Beckmann, M.J. Kemper, D. Nolkemper, R. Ganschow, M. Burdelski. Departments of Pediatrics[1] and Hepatobiliary Surgery[2], University Medical Center Hamburg-Eppendorf, Germany.

In primary hyperoxaluria type 1 (PH 1), deficiency of hepatic alanine glyoxylate aminotransferase (AGT) leads to renal calcium oxalate deposition with urolithiasis and nephrocalcinosis with a high risk of renal failure. Because preemptive liver transplantation (PLTx) curses the metabolic defect in PH 1, it might be considered early to prevent end-stage renal disease (ESRD). In a retrospective analysis we present the long-term follow-up of four patients, who underwent PLTx in our center from 1995 to 1996.

Patient	Age at PLTx	GFR at PLTx	Follow-up
1	3,3	82 ml/min/1,73m^2	Normal liver tests, Tacrolimus (TAC) because of hirsutism, GFR 96 ml/min/1,73m^2, 9 years post LTx
2	6	98 ml/min/1,73m^2	Normal liver tests, TAC and Cellcept for 1,5 years because of chronic rejection caused by non compliance. GFR 111ml/min/1,73m^2, 9 years post LTx
3	2,8	54 ml/min/1,73m^2	Normal liver tests, GFR 62 ml/min/1,73m^2, 7 years post LTx Switch from CsA and Azathioprine to Rapamune, GFR improved up to 76 ml/min/1,73m^2 1 year later
4	9,8	27 ml/min/1,73m^2	ESRD, hemodialysis 6 years after Tx for 2 years followed by kidney transplantation, Normal liver tests 8 years after LTx

PLTx could be a promissing procedure for the treatment of PH1 preventing the development of ESRD in these patients and it should not be delayed too long (eg. GFR 40-50). With improving medical treatment of PH1, the optimal timing of PLTx, however, is a matter of debate, which may be resolved by further prospective studies.

N° 7

MUTATION ANALYSIS OF THE G6PT GENE IN RUSSIAN PATIENTS WITH GSD1B : IDENTIFICATION A NOVEL MUTATION

E. Lomonosova, N. Povalko. Department of Inherited Metabolic Diseases, Research Centre for Medical Genetics, Russian Academy of Medical Sciences, Moscow, Russia.

Glycogen storage disease type 1b is caused by mutations in the glucose 6-phosphate translocase (G6PT) gene that is composed of nine exons spanning genomic region of approximately 4 kb. To date, over 70 G6PT mutations have been identified. Clinically, GSD 1b is characterized by severe fasting hypoglycemia, gepatomegaly, neutropenia and impaired neutrophil function, particularly in relation to gram-positive organisms.
Two Russian patients from two unrelated families with classical clinical picture of GSD 1b were tested for mutations in the G6PT gene by PCR and single strand conformation polymorphism (SSCP) analysis. Subsequently, automated DNA sequencing was used to verify the mutated fragments. For each identified mutation, a restriction fragment length polymorphism (RLFP) assay was performed.
Results and Conclusions : Two deletions were found in G6PT gene : 1041-1042delCT and 335-356del22. One patient has genotype1041-1042delCT/1041-1042delCT and another 1041-1042delCT/335-356del22. Mutation 335-356del22 predicting a shift the reading frame and formed a truncated nonfunctional protein was not previously published. The present results allow us to enlarge our knowledge about nature genetic defects in G6PT gene and help in rapid and non invasive diagnosis of this disease

N° 8

NEONATAL HEMOCHROMATOSIS : 12 YEARS EXPERIENCE OF A LIVER TRANSPLANT CENTER

E. Grabhorn[1], A. Richter[1], D. Nolkemper[1], M. Burdelski[1], X. Rogiers[2], R. Ganschow[1]. (1) Departments of Pediatrics and (2) Hepatobiliary Surgery, University Medical Center Hamburg-Eppendorf, Germany.

Background : Neonatal hemochromatosis (NH) is a severe, often lethal multiorgan disorder of the iron metabolism, the liver is the predominantly affected organ. The aetiology is still not clearly determined yet. Liver transplantation can be a curative therapy, the benefit of antioxidant treatment is discussed controversially in literature. We summarize our experience with NH in the past 12 years.
Patients : We analysed 15 patients, who were admitted to our institution form 1992 to 2004 with acute liver failure because of NH. All patients were presented within the first 30 days after birth (rang : 1 to 30 days ; median : 2 days).
Results : Median weight at the time of diagnosis was 2900 g (range :1640-3890 g), 7/15 patients (46,6%) were small for gestational age, 6/15 patients (40%) premature infants. All patients had elevated ferritin levels (median : 1868 µg /l), and transferrin saturation (median : 110%), moreover a reduced transferrin level was found (median : 1,2 g/l). Fourteen patients (93%) were diagnosed by iron overload in hepatocytes in biopsies, 3 had further iron deposition in minor salivary glands, pancreas or heart. One patient was diagnosed by MR imaging. Seven infants were transplanted (46,6%), 6 of them in combination with a preceding antioxidant treatment. Of these, all but one child survived. Three children received an antioxidant cocktail without the necessity of LTx and are fine up to now. Five patients (33,3%) died, 3 of them without any treatment because of initial fulminant multiorgan failure.
Conclusions : NH is a severe metabolic disease, but early antioxidant treatment together with LTx and optimal medical care can improve the outcome. Children with moderate liver failure can survive without LTx only by antioxidant treatment, but should be monitored closely for deterioration.

N° 9

LIVER TRANSPLANTATION IN METABOLIC DISEASES : THE EXPERIENCE OF A METABOLIC UNIT

R. Parini[1], F. Furlan[1], F. Santus[1], M. Rigoldi[1], F. Menni[2], G. Rossi[2], G. Nebbia[2], M. Colledan[3], P. Tagliabue[4], L. Ventura[4], M.L. Melzi[3], P. Stroppa[3], G. Torre[3], D. Codazzi[5]. (1) Centro «Fondazione Mariani per le Malattie Metaboliche dell'Infanzia» and (4) Neonatal intensive care Unit, S. Gerardo Hospital, Universita Milano Biocca, Monza ; (2) Dept. of Pediatrics and Transplant Unit, H Maggiore Policlinico, IRCCS, Milano ; (3) Liver Transplant Unit and (5) Pediatric Intensive Care Unit, Ospedali Riuniti Bergamo.

AIM : retrospective review of all patients (pts) evaluated for liver transplantation (OLT) in our center from 1985 to 2005. Out of a total of 167 patients (38 organic acidosis, 58 aminoacidopathies, 45 urea cycle disorders, 26 liver glycogenosis, 25 mitochondrial disorders), we considered liver transplant as the best therapeutic goal for 15 patients and transplanted 8.
1-Liver glycogenosis (GSD) : female GSD Ia renal insufficiency at 16y. She had combined OLT and kidney transplantation at 19. Uneventful pregnancy after 2 y and now, 4 y later normal liver and kidney function and free diet**. 2-Organic acidosis (OA)** 14 pts died for actue decompensation after 2-16 y of dietetic treatment. 5 had indication for OLT : 2 of them died before beingtransplanted and 3 underwent OLT at the age of 6y, 12 and 8 mo. The pateints are well after 5 years, 4 and 6 mo respectively. **3-Aminoacidopathies (AA)** 2 pts with Tyrosinemia type I underwent OLT, one patient at 20 mo of age before the treatment with NTBC was available : the second at 3 mo of age, for hepatic failure, despite NTBC has been started since 2 weeks**. 4-Urea cycle disorders (UCD) :** one late-onset OTC female, who had frequent decompensations despite the dietetic and thearpeutic treatment and was transplanted age 17. She is now 31 y old and is in good health ; c) one neonatal citrullinemia who is at present waiting for OLT. **5-Respiratory chain defects (RCD)** one patient with a liver limited RCD underwent OLT at 2 y and is now in good health at the age of 4 y.
CONCLUSIONS : indications for OLTare still limited in metabolic diseases. We do not suggest OLT for well compensated patients who are the majority of GSD, AA and UCD. OA, considering their poor long-term prognosis, are in our opinion those who could mainly benefit of OLT, although we are concerned about the persistence of toxic aycl-CoA production from the muscles.
Acknowledgements : Fondazione P e L Mariani - Milano

N° 10

HEPATIC TRANSPLANTATION IN METHYLMALONIC ACIDAEMIA : A CASE REPORT

Fuoti M., Pinotti M., Villa M.C., Celano M.R., Miceli V., Amoruso C., Rossi G., Parini R., Nebbia G. Clinica Pediatrica e Centro Trapianti Fegato, Fondazione Policlinico, Mangiagalli, Regina Elena. Milano-Italy.

Mut0 methylmalonic acidaemia (MMA) was diagnosed in an Italian boy at few months of life. Since the 3rd year of life, a worsening of the metabolic equilibrium appeared, despite low protein diet, nocturnal enteral tube feeding and supportive therapy. Frequent episodes of metabolic decompensation required a great number of hospital admissions. Progressive renal insufficiency emerged. Hepatic function appeared preserved on all occasions and any neurological complication did not occur. At the age of 8 years and 2 months reduced size hepatic transplantation (LTX) was performed, without any relevant complication. Immediate amelioration of clinical conditions was noticed. The boy is now 14 years and neither metabolic decompensations with acidosis nor neurological problems have appeared without any nutritional treatment within almost 6 years. The number of hospital admissions greatly decreased and we observed also a progressive and significant reduction of methylmalonate levels in urine (mean values : preLTX : 9766 ± 750 SD vs postLTX : 4976 ± 506 SD mmol/mol creatinine; significant t test with p value : < 0.001). Nephropathy showed a steadying after LTX, with a creatinine clearance of 75.69 ml/min and a plasmatic creatinine of 1.09 mg/dl at present. We cannot conclude however there was a significant improvement of renal function after LTX. Even growth has shown an important resumption, passing from greatly under the 3rd percentile for age at the time of transplantation to over the 25th percentile.
Concluding, LTX can be considered as a possible treatment for selected patients with MMA associated to an important improvement of the quality of life.

N° 11

EXPERIENCES WITH NTBC AND LIVER TRANSPLANTATION IN TYROSINEMIA TYPE I IN THE NETHERLANDS

Francjan J. van Spronsen[1], L.M.J. Pierik[2], H.J. Verkade[3]. (1) Section of Metabolic Diseases, (2) Section of Nephrology, (3) Liver transplantation Team, Beatrix Children's Hospital, University Medical Center of Groningen, University of Groningen, Groningen, The Netherlands.

Tyrosinemia type I results in liver failure, hepatocellular carcinoma (HCC), renal tubular dysfunction, and acute intermittent porphyria. Till 1992 the most important question was when to perform OLT. From 1992, most patients have been treated with 2-(2 nitro-4-3 trifluoro-methylbenzoyl)-1,3-cyclohexanedione (NTBC) preventing the formation of the pathological products of tyrosinemia type I by inhibition of an enzyme located upstream from the primary enzyme defect in liver and kidney. This NTBC cures liver failure, renal tubular dysfunction, and acute porphyria. In addition, NTBC is reported to prevent HCC when started < 2 years of age (Holme, *Clin Liver Dis*, 2000, **4** : 805-14). In the Netherlands, however, development of HCC occurred after a long period of NTBC (van Spronsen, *J Pediatr Gastroenterol Nutr*, 2005, **40** : 90-3).

Till now, we have found HCC in 4 patients, 2 starting with NTBC < 1 year of age. Compared to other patients, these 4 with HCC had either a very high AFP at the start of treatment, a slow decrease, no complete normalization or a real increase after normalization. Therefore, all these may be of predictive value for HCC.

Our experiences after OLT in 9 patients with tyrosinemia type I shows some tubular problems (increased loss of phosphate and calcium) that may be progressive. The patient with the most severe tubular deterioration was also the patient with the highest excretion of succinylacetone. This suggests an ongoing toxic effect of tyrosinemia type I in the kidney, that may need NTBC again. Therefore, further studies are necessary on both the early and late risk of HCC in early NTBC treated patients and on renal (especially tubular) function after OLT in these patients.

N° 12

CRYOPRESERVATION OF MURINE HEPATOCYTES INDUCES MITOCHONDRIAL MEMBRANE POTENTIAL ALTERATION WITHOUT INDUCTION OF CASPASE 3 ACTIVITY

X. Stéphenne[1], B. Guigas[2], M. Najimi[1], L. Hue[2], E. M. Sokal[1]. Université Catholique de Louvain, (1) Laboratoire d'hépatologie pédiatrique et de thérapie cellulaire, (2) Unité Hormones et Métabolisme.

Aim of the work : Transplantation of cryopreserved liver cells has recently been demonstrated as a safe alternative able to correct partially inborn errors of metabolism. The use of cryopreserved cells allows planning multiple infusions. The aim of this study was to evaluate the effects of cryopreservation on the quality of isolated hepatocytes, as cryopreservation has been reported to induce cellular damage by a pathway that can involve the mitochondria.

Material and Methods : Mice hepatocytes were isolated according to a double-step collagenase perfusion. Cells were then used immediately or cryopreserved by a slow freezing protocol in a medium containing UW solution, 25% FCS and 10% DMSO. Before and after cryopreservation/thawing, cell viability was estimated by the trypan blue exclusion test. Intracellular ATP concentrations, mitochondrial deshydrogenase activity (MTT test) and the extracellular release of LDH were also determined. The oxygen consumption rate (JO_2) and the mitochondrial membrane potential ($\Delta\Psi$) were measured with Clark electrode and the $\Delta\Psi$-mediated mitochondrial uptake of Rhodamine 123 respectively. The cytosolic release of cytochrome c and caspase-3 activity were also investigated in both fresh and cryopreserved/thawed cells.

Results : Viability of cells decreased following cryopreservation (52 ± 4 *vs* 92 ± 1% in fresh hepatocytes, $p < 0.05$) and a dramatic drop of ATP concentration was evidenced (0.01 ± 0.0 *vs* 0.4 ± 0.2pmol/cell in fresh cells, $p < 0.05$). No significant differences were observed for the MTT test and the extracellular release of LDH between fresh and cryopreserved/thawed cells, indicating an apparent normal activity of the Krebs cycle and no increase in cell membrane permeability. After cryopreservation/thawing, the basal JO_2 was slightly decreased as compared to fresh cells (20.4 ± 3.4 *vs* 29.3 ± 3.2nmoles O_2/min/10^6 cells in fresh cells, $p = 0.1$). However, addition of 2,4-Dinitrophenol, an uncoupler of mitochondrial oxidative phosphorylation, increased the JO_2 in fresh but not in cryopreserved/thawed cells, suggesting an alteration of the mitochondrial machinery. Indeed, the mitochondrial membrane potential was significantly reduced after cryopreservation (88 ± 17 *vs* 288 ± 41A.U. in fresh cells, $p < 0.05$). The pro-apoptotic protein cytochrome c, which is absent in the cytosol of fresh cells, was detected in the cytosolic compartment after cryopreservation. However, the activity of the caspase 3, located downstream of cytochrome c in the mitochondria-mediated apoptotic pathway, was surprisingly not modified in cryopreserved cells (2830 ± 1561 *vs* 2978 ± 799A.U. in fresh cells).

Conclusions : Our results indicate that cryopreservation of murine hepatocytes induce modification of the mitochondrial machinery leading to a drop in $\Delta\Psi$ that could explained the dramatic decline of ATP levels. Although cryopreservation induces cytosolic release of cytochrome c, no difference of caspase 3 activity was detected between fresh and cryopreserved/thawed cells, indicating that cells were possibly not committed to an irreversible apoptotic program.

N° 13

PREVENTIVE TREATMENT OF RENAL INSUFFICIENCY WITH HYPERHYDRATION IN 10 CHILDREN WITH HYPEROXALURIA TYPE I (HP I)

M.F. Gagnadoux, R. Salomon, F. Lacaille, B. Boudaillez, S. Taque, JP Bertheleme, C. Guyot, P. Niaudet. Hôpital Necker-Enfants malades, Paris, CHU d'Amiens, Centre Perharidy de Roscoff, CHU de Nantes, CHU de Rennes, France.

Hyperoxaluria type I is caused by the hepatic defect in alanine :glyoxylate aminotransferase, leading to hyperproduction of oxalate, deposition of calcium oxalate in the kidney and renal insufficiency through nephrocalcinosis. Once renal insufficiency is established, the only effective treatment is combined liver and kidney transplantation. But when HP I is diagnosed at a pre-symptomatic stage, permanent dilution of urines through hyperhydration could prevent the deposit of calcium oxalate, thus the kidney destruction. We treated 10 children, 6 of whom had lost 1 sibling of HP I ; 2 were treated from birth, 8 at 4 to 12 years of age. 6 had renal stones, 4 nephrocalcinosis (2 + stones), 2 were asymptomatic. Glomerular filtration (GF) was < 60 ml/mn/1,73 m^2 in 2, 60-80 in 2, > 80 in 6. Hyperhydration was ≥ 3 l/m^2/day water, oral or through a gastrostomy in 5, a nasogastric tube in 2. Sodium citrate, bicarbonate and magnesium were given as cristallization inhibitors. Results : mean follow-up was 5.5 years (1-10). Two patients developed end-stage renal failure after 6 (pregnancy) and 10 (immobilization due to car accident) years. One with poor complicance had chronic renal failure with GF 48 at 9 years. 7 had a GF > 80 with 1-8 years FU (mean 4 y). Nephrocalcinosis was not observed in the 2 treated from birth (FU 6 and 8 years) ; it was moderate and stable in 1 ; stones were stable or diminished in 6, and appeared in 1 non-compliant patient. Conclusion : permanent and life-long dilution of urines can delay or even prevent nephrocalcinosis and thus renal failure, especially when initiated early. The limitation is the compliance of the adolescent and the adult, as any episode of dehydration can precipitate nephrocalcinosis. However this non-invasive treatment compares favorably to pre-emptive liver transplantation proposed before the developement of renal failure as an alternative to combined liver and kidney transplantation.

N° 14

TWELVE YEARS FOLLOW-UP OF LIVER TRANSPLANTATION (LT) FOR PROPIONIC ACIDEMIA

F. Lacaille, J. Laurent, P. de Lonlay, R. Salomon, J.M Saudubray. Department of Paediatrics, Hôpital Necker-Enfants malades, Paris, France.

A girl with propionic acidemia was evaluated for LT at age 8 because of frequent decompensations. She had a moderate cardiomyopathy, with a moderetely dilated and hypokinetic left ventricle (ejection fraction 30%). Height was –2,5 SD. The girl received LT at age 9, in 1993, with ciclosporine. Early complications were rejection, CMV hepatitis, diabetes needing insulin 1.5 months, post-transfusion hepatitis B despite adequate vaccination. No metabolic decompensation was observed. Later evolution was : 1- growth and development : final height was 146 cm. She began to work as a secretary. 2- Liver : the hepatitis B was treated with lamivudine from 1996, with HBe sero-conversion and DNA negativation. Because of mild rejection, tacrolimus was introduced in 1999. ALT were normal, but GGT elevated (300-400 IU). On the biopsy 10 years post-LT, there was a decreased number of bile ducts without true paucity. Treatment was tacrolimus (blood level 5), 0.2 mg/kg prednisone on alternate days, and ursodeoxycholic acid. 3- Kidney : renal dysfunction was noted from one year post-LT. It was stable with a creatinine of 150 µM. Small cysts were noted in both kidneys. Renal biopsy was not performed because of increased bleeding time and cysts. Blood pressure was controlled with nifedipine and enalapril. 4- Heart : mild left ventricle hypertrophy persisted, contractility was normal. 5- Bone : during growth she had back pain and had to wear a corset. Densitometry showed a mild ostoporosis. 6- The only biological abnormality was excretion of methylcitrate (metabolite with no known toxicity) in the urines. Conclusion : the role of the original disease on the renal dysfunction, that may be due to the drugs, or on the final height, is uncertain. The cardiomyopathy improved. We did not observe symptoms that could be due to the persistent metabolic defect in extra-hepatic organs. We believe that, despite the problems described in this young woman, LT is a valuable treatment option for this severe metabolic disease. It has to be performed before the development of significative cardiomyopathy or neurological problems secondary to metabolic decompensations.

N° 15

PRE-CLINICAL APPROACH OF HEPATOCYTE TRANSPLANTATION IN NON-HUMAN PRIMATES : EFFICIENT HEPATOCYTE ENGRAFTMENT AFTER PARTIAL PORTAL EMBOLISATION

I. Dagher[1,2], L. Boudechiche[1], J. Branger[1], A. Coulomb[3], M. Hadchouel[1], M. Andreoletti[4], D.Pariente[5], D Franco[1,2], A. Weber[1]. (1) EMI 00-20 and (2) Department of Surgery, Antoine Beclere Hospital, Clamart, (2) Inserm EMI 0020, Bicetre Hospital, Kremlin-Bicêtre, (3) Pathology, (4) Anestesiology, Antoine Beclere Hospital, Clamart, (5) Radiology, Bicêtre Hospital, Kremlin-Bicêtre, France.

Hepatocyte transplantation is becoming an alternative to orthotopic liver transplantation for the treatment of life-threatening metabolic diseases. In rodents, transplanted hepatocytes repopulate efficiently recipient livers only when resident hepatocytes are destroyed or when their proliferation is blocked. These models are not transposable to humans. Partial portal embolisation (PPE) is currently used to induce liver regeneration in humans. The **aim** of our study was to define conditions for hepatocyte transplantation after PPE and to evaluate the efficiency of hepatocyte engraftment in a large animal model closely related to humans. Eight Macaca mulatta were used. The hepatic left lateral lobe representing 20% of the liver was removed and used for hepatocyte isolation by a two-step collagenase perfusion. The left portal branch and the right anterior branch were embolised with a biological glue (histoacryl®). The proliferation of resident and transplanted hepatocytes was measured by BrdU incorporation on liver biopsies on days 3, 5 or 7 after embolisation. Four hundred millions hepatocytes (4% of total hepatocytic mass), were isolated, labelled with Hoechst fluorescent nuclear dye, then immediately transplanted. Engraftment of transplanted hepatocytes was assessed by immuno-histochemistry. PPE induced significant liver regeneration and as soon as 3 days after embolisation 23% of hepatocyte were proliferating and 10% were still dividing on days 5 and 7 in non-embolised segments. Transplanted cells proliferated, and represented 10.9 ± 0.7% of the monkey liver at day 7 and their number remained stable 15 days after transplantation suggesting that they had participated to liver regeneration. Expression of connexin 32 between transplanted and resident hepatocytes demonstrated that transplanted cells were integrated and were functional. Long-term biopsies (9 months) revealed a normal architecture and the absence of nodular regenerative hyperplasia. Our **results** suggest that partial portal embolisation of 50% of the liver performed prior to hepatocyte transplantation is a safe procedure to achieve efficient cell engraftment in a model of autologous hepatocyte transplantation. PPE is potentially suitable for use in patients and 10% liver repopulation should be sufficient for the treatment of metabolic diseases.

N° 16

EFFECT OF GLYCERIL TRINITRATE ON HEPATOCYTES ENGRAFTMENT AND METABOLIC CORRECTION IN THE MDR2 -/- MOUSE MODEL OF PROGRESSIVE FAMILIAL INTRAHEPATIC CHOLESTASIS

Lyes Boudechiche, Ibrahim Dagher, Anne Spraul, Anne Weber, Dominique Franco, and Michelle Hadchouel. Laboratoire de transfert de gène dans le foie, INSERM EMI 00-20 - hadchouel@kb.inserm.fr

Background and aims : Althought 50 cases of clinical hepatocyte transplantation have been documented, therapeutic benefit is still insufficient, probably due to insufficient number of engrafted hepatocytes, most of the patients have to benefit later from orthotopic liver transplantation. We studied wether hepatic sinusoidal dilatation with glycerol trinitrate will enhance hepatocyte engraftment in the mdr2-/- mouse model in which lack of biliary phospholipid secretion leads to liver disease.
Methods : Normal mdr2(+/+) hepatocytes were isolated and transplanted in mdr2 (-/-) mice model concomitantly with glyceril trinitrate infusion. Liver repopulation was assessed by immunohistochemistry and measurement of biliary lipid secretion.
Results : Transplanted hepatocytes repopulated the hepatic parenchyma and diminished liver pathology. Glyceril trinitrate improves hepatocyte engraftment and phospholipid secretion 3,6 fold compared to control transplanted animals (18 versus 5 nmol/min/100g).
Conclusion : These results suggest that sinusoidal vasodilatation benefited intrahepatic distribution of transplanted cells and enhances metabolic correction after cell transplantation. This therapy is potentially applicable to patients with PFIC type 3.

N° 17

A 2,3 KG LIVER RECIPIENT FOR NEONATAL HEMOCROMATOSIS : A CASE REPORT

M. Bosisio, P. Stroppa, M. Candusso, M. Bravi, M.L. Melzi, G. Torre, M. Colledan. Pediatric liver transplantation Centre-Ospedali Riuniti-Bergamo-Italy.

Neonatal hemochromatosis (NH)is a very rare disorder in which liver injury of fetal onset is associated with massive iron deposition, sparing the reticuloendothelial system, characterized by neonatal acute liver failure with coagulopathy, hypoalbuminemia, hypoglycemia and jaundice often occurring from the first day of life. The etiopathogenesis is unclear, distinct from hereditary hemocromatosis, NH may not be due to a single etiology but rather to the ultimate phenotype of different causes (genetic, autoimmune, infective). There is a high recurrence rate within families. The prognosis is extremely poor and the diagnosis is often based on iron deposition at autopsy. The efficacy of treatment with antioxidant/chelator cocktail is controversial and it seems to be effective if early administered to patient with mild disease. Liver transplantation is considered the treatment of choice for severe cases with no improvement with supportive medical treatment. This terapeutical option is limited by the small size of the patients and the difficulties to find an appropriate donor. We report a successful case of a 2,3 Kg newborn with NH who underwent liver transplantation. A male delivered at 34 GW by caesarian section for intrauterine growth retardation, oligoidramnios, foetal ascites, body weight 2280 grams, lenght 42 cm. In the first hours of life severe liver failure occurred with coagulopaty, hypoalbuminemia, hypoglicemia, jaundice and respiratory failure due to abdominal distension which needed paracentesis. Studies for inherited metabolic disorders and infectious diseases were no diagnostic. The diagnosis of NH was suggested by the iron panel and was confirmed by the liver biopsy performed at 20 days of life. Magnetic resonance imaging didn't show iron deposition in other organs. Antioxidant/chelator cocktail therapy was initiated and because of the severe liver failure the baby underwent orthotopic liver transplantation (OLTX) at 45 days old. He received a 200 grams left graft from a 20 Kgs donor. Because of acute renal failure the immunosuppressive regimen was started with steroid and basiliximab, tacrolimus was added just after 11 days. Posttransplant course was characterized by respiratory difficulties due to hepato-spenomegaly, CMV infection with pneumonia and hepatitis, sepsis from abdominal biliary collection. The baby was discharged 113 days after the transplant. After 39 months the growth pattern is within normal range, the liver function is normal, the liver biopsy doesn't show iron deposition.. Conclusions : newborns with suspected NH must be precociously referred to a pediatric liver transplantation centre, because OLTX must be considered as soon as it becomes apparent that medical support is useless, before any neurological complications. Specific pediatric expertise is required for these patients : the small size of recipient is strongly related to high mortality and morbidity rate, due to the low number of matched size donors and to technical and infective post OLTX complications. In our experience this is the smallest infant we have so far transplanted even if, in all cases, 57% of patients (145 out of 255) weighted below 10 Kg.

N° 18

LIVER TRANSPLANTATION IN CHILDREN WITH METABOLIC AND GENETIC DISORDERS : EXPERIENCE OF ONE CENTER

P. Stroppa, M. Bosisio, M. Bravi, M.L. Melzi, M. Candusso, G. Torre. Pediatric liver transplantation Center-Ospedali Riuniti-Bergamo-Italy.

Metabolic and genetic diseases represent an heterogeneus group of disorders with liver involvement and in our centre have been the second largest indication for orthotopic liver transplantation in children after extrahepatic biliary atresia (EHBA). The clinical presentation of liver disease could be heterogeneous, ranging from chronic end-stage liver failure to fulminant hepatic failure. In many patients enzymatic deficiency leads to damage in other organs (kidneys, heart, central nervous system), and is potentially complicated by hepatocellular carcinoma. The aim of this study is to retrospectively review our experience with orthotopic liver transplantation for metabolic/genetic disorders in children. Between october 1997 and june 2005, 288 liver transplantation were performed in 255 children. 57 children with metabolic/genetic disease (22.4% of total number of transplanted children) underwent 64 transplants. Indications included an heterogeneus group of diseases : 24 AGS, 13 Byler disease, 3 CNS-1, 2 familiar hemolytic uremic syndrome (SEU), 2 neonatal hemochromatosis, 2 glicogenosis IV, 2 Wilson's disease, 1propionic acidemia, 1methylmalonic acidemia, 1 tyrosinemia, 1 ornithine transcarbamylase deficiency (OTC), 1 cystic fibrosis, 3 congenital hepatic fibrosis. The diagnosis was mostly based on clinical manifestation, blood tests, strumental examinations and on hepatic biopsy. When possible genetic analysis was performed to complete the diagnostic procedure. There were 32 males and 25 females with a median age at the transplant of 2.36 years (r.29 days to 16.78 years). The median weight was 10 Kg (r.2.3 to 55 Kg). The median waiting time was 37 days (r.1 to 255 days). Every patient received liver from a cadaveric donor. The split

liver technique was performed in 42 cases, whole liver in 18 cases and reduced liver in 3 cases. Indication for combined kidney and liver transplantation included hyperoxaluria(1 case), familiar SEU(2 cases), congenital hepatic fibrosis (2 cases). Retransplant was required in 7 cases (12.3%). The overall survival rate (OSR) is 89% at 1 year, 86% at 3 years (OSR of EHBA is 92% at 1 year). Six patients died. The main medical complications were represented by acute (11 cases, 19.3%) and chronic (10 cases, 17.5%) rejection, de novo autoimmune hepatitis(4 cases), bacterial and viral infections and PTLD (1 case). Biliary complications were reported in 11 patients (19.3%), vascular complications in 2 patients (portal vein thrombosis). Neurological complication in one case of SEU. Liver transplantation represents an effective method of replacing a failing liver, and corrects the underlying defect in many cases. The clinical phenotype of all our patients was corrected and metabolic decompensation did not reoccur after OLTX. Liver transplantation should be considered as a promising treatment approach currently available to improve quality of life. The mortality rate and the complications of this group of patient are mostly comparable to that of the other group of transplanted children.

N° 19

ENDODERMAL PROGENITORS FROM SALIVARY GLAND FOR REPOPULATION OF LIVER AND TREATMENT OF METABOLIC DISORDERS

Endo F., Hisatomi Y., Okumura K., Matsumoto S., Satoh A., Hattori K., Nakamura K. Department of Pediatrics, Graduate School of Medical Science, Kumamoto University, Honjo 1-1-1, Kumamoto 860-8556, Japan.

In the salivary gland, tissue damage induced by ligation of main ducts leads to the disappearance of acinar cells and marked proliferation of ductal cells. Reopening of the ducts triggers the repopulation of acinar cells within 1 to 2 weeks, which suggests activation of tissue progenitor cells in a damaged state. We isolated possible progenitors from salivary glands and designated the cells as SGP-1 cells. SGP cells formed clusters on type I collagen-coated dishes and differentiated into endodermal lineage. Two major types of clusters appeared : one containing cells positive for AFP and/or albumin (hepatic cluster) and the other positive for glucagon and/or insulin (pancreatic cluster). On laminin-coated dishes, SGP-1 selectively differentiated into hepatic type cells. When the cells were transplanted into the liver of LEC rats (a model for Wilson disease), they partially repopulated. Using immunohistochemistry and flow cytometry, we sorted the Sca-1(+)/c-Kit(+) fraction from adult mice salivary glands by way of fluorescence-activated cell sorting. The sorted cells were apparently homogeneous and were designated mouse salivary gland-derived prgenitors (mSGPs). When spheroidal bodies of mSGP, 20 to 30 μm in diameter were transplanted into liver via the portal vein, the cells were integrated into the hepatic cords and expressed albumin and alpha1-antitrypsin, suggesting that they had differentiated into hepatic-type cells. Moreover, duct-like structures formed by mSGP cells also eppeared, epithelial cells positive for cytokeratin 19. These results suggest that the salivary glands are a good candidate as a source of endodermal progenitors for cell therapy.

N° 20

IS LIVER TRANSPLANTATION INDICATED IN METABOLIC DISORDERS WITH NEUROLOGICAL INVOLVEMENT ? TWO CASE REPORTS

P. Verloo, L. De Meirleir, R. Van Coster, S. Seneca, J. Smet, S. Van Biervliet, M. Van Winckel. University Hospital Ghent, Belgium.

Patient 1, a 3-year-old girl, was admitted to the hospital with metabolic decompensation, lactic acidosis, pancreatic insufficiency and sideroblastic anaemia. The diagnosis of Pearson disease was made using southern blot of mitochondrial DNA. Her metabolic crises could only be controlled using parenteral nutrition and continuous administration of high doses of intravenous bicarbonate. We considered liver transplantation since it might diminish the episodes of severe lactic acidosis. However cerebral MRI demonstrated leuco-encephalopathia and cytotoxic edema. Additional proton spectroscopic cerebral MRI suggested that the cytotoxic edema was caused by local production of lactate and was not the consequence of general metabolic decompensation. Moreover, respiratory chain enzyme activities were decreased in liver as well as in muscle tissue. Since these investigations showed extensive extra-hepatic involvement, it was decided, in agreement with the parents, to restrain from liver transplantation.

Patient 2 is a 7-year-old boy with HHH (hyperammoniemia, hyperornithinemia, homocitrullinuria). He was originally admitted to the hospital at the age of two months with severe hyperammoniemia. He recovered from the acute phase, but had severe neurological sequelae (mental retardation). Moreover, even though his protein intake was strictly controlled, he continued having severe metabolic crises with hyperammoniemia for which repeated hospitalisations were and are necessary. In order to avoid these hospitalisations and in order to be able to allow a less restricted diet, liver transplantation was considered a therapeutic option. The parents were informed repeatedly and extensively on risks and complications of liver transplantation and inherent immunosuppression therapy, and on the fact that no former experience with liver transplantation for this condition has been reported. Fully informed, they remain strongly in favour of transplantation. So, despite his mental retardation, he is currently waiting for a suitable liver.
These two case reports demonstrate how neurological complications of metabolic disease influence decisions on liver transplantation. Liver transplantation is an option when the liver is the primary source of metabolic disturbances. New techniques such as MRI spectroscopy are helpful in demonstrating the difference between primary or secondary neurological damage. Even when neurological damage is present, liver transplantation remains an option capable of enhancing the quality of life in patients with metabolic disease, but requires careful consideration.

N° 21

PRIMARY HYPEROXALURIA TYPE ONE - CAN FOETAL LIVER BIOPSY AND LATER HEPATORENAL TRANSPLANT STILL BE THE TREATMENT OPTIONS ?

Dr A. Patwardhan James Cook University Hospital Middlesbrough, Dr. C. Higgins. Royal Wolverhampton. Place were work Done- Royal Wolverhampton

Introduction : Primary Hyperoxaluria type 1 is a rare autosomal recessive, Peroxisorhal disorder. To our knowledge, no published data is available on onset in foetus presentation ; Definitive diagnosis only possible by liver biopsy and assay of AGT enzyme activity.
Birth History : A term male baby, born by emergency caesarean section, for maternal reasons, to Asian parents, with parental consanguinity. Uneventful antenatal history, no risks factors for infection. The baby presented at 5 hours of age with dusky episodes. He was hypotonic with unexplained Techypnoea and Tachycardia. After excluding septic chromosomal, muscular and metabolic causes, diagnosed as Primary Hyperoxaluria type 1. The diagnosis was based on urinary oxalate, glycolates level, increased oxalate creatinine ratio, renal calcinosis, and echo dense parenchyma on renal ultrasound. The CT scan- brain showed left middle cerebral arterial infarct with unilateral enlargement of ventricle and left porencephalic cyst, inferred by consultant radiologist as an antenatal event. Tachycardia and Techypnoea denoted probable cardiac involvement. Baby improved on diuretics, water supplementation, Pyridoxine, Prophylactic Trimethoprim and Albright solution.
Conclusion : Presentation of PH1 in intrauterine life is an unknown event till date. Antenatal diagnosis is not easy since blood and amniotic fluid biochemical tests are non-conclusive in foetus and definitive diagnosis needs AGT enzyme activity detection therefore foetal liver biopsy and assay. The question is does the confirmation of the diagnosis by foetal liver biopsy change the management in high risk groups ? Should the foetal liver biopsy be offered to these babies at all ?
Implication and issues : This case raises two issues, first is if such children be given a hepatorenal transplant a treatment option ? and the other is whether any treatment is possible for the foetus (intra uterine) if diagnosed early in pregnancy. The management of primary Hyperoxaluria type 1 in foetus will be a major example of the ethical, epidemiologic, technical, and financial challenges that will rise specially in developing world which has the highest incidence of the condition but negligible therapeutic options. In certain circumstances, oxalosis can be regarded as a condition for which therapeutic withdrawal may be an acceptable option. Should late abortions be offered as an option ?